6

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: October 27, 2010

SUBJECT: Ethylenethiourea (ETU): Review of Rabbit Developmental Toxicity Study

PC Code: 600016
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0055337
MRID No.: 47976403, 47976401, 47976402

DP Barcode: D376196
Registration No.: GPCI- 014504-26952
Regulatory Action: N/A
Case No.: N/A
CAS No.: 9006-42-2
40 CFR: N/A

Ver.Apr.08

FROM: Kit Farwell, D.V.M.
Risk Assessment Branch VII
Health Effects Division (7509P)
Office of Pesticide Programs

THROUGH: Michael Metzger, Branch Chief
Risk Assessment Branch VII
Health Effects Division (7509P)
Office of Pesticide Programs

TO: Christina Scheltema, Chemical Review Manager
Pesticide Re-evaluation Division (7508P)
Office of Pesticide Programs

Attached is the review for a developmental toxicity study in rabbits with Ethylenethiourea (ETU).

Main study: Munley, S.M. (2010). Ethylenethiourea (ETU): Developmental Toxicity Study in Rabbits. E .I. du Pont de Nemours and Company, Newark, Delaware. Laboratory report number: DuPont-17807-843. January 14, 2010. Unpublished. MRID 47976403.

Rangefinding studies: Munley, S.M. (2010). Ethylenethiourea (ETU): A 14-Day Tolerability Study in Non-Pregnant Rabbits E. I. du Pont de Nemours and Company, Newark, Delaware. Unpublished. MRID 47976401.

Received in RAC 10/28/2010 EW

Munley, S.M. (2010). Ethylenethiourea (ETU): Pilot Developmental Toxicity Study in Rabbits. E.I. du Pont de Nemours and Company, Newark, Delaware. Unpublished. MRID# 47976402.

Conclusions: The NOAEL for maternal toxicity is 15 mg/kg/day. The LOAEL for maternal toxicity is 50 mg/kg/day, based on reduced body weight gain of -30%, which corresponded to a decrease of -4% for absolute body weight. Additionally, a lowered food consumption of -14% occurred over the length of the study.

The NOAEL for developmental toxicity is 15 mg/kg/day. The LOAEL for developmental toxicity is 50 mg/kg/day based upon reduced fetal weights. Also occurring at this dose were domed heads (2 pups) and hydrocephaly (1 pup).

This developmental toxicity study in the rabbit is classified ACCEPTABLE/GUIDELINE and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700b; OECD 414) in rabbits.

DATA EVALUATION RECORD

Ethylenethiourea
PC Code:600016
TXR#: 0055337
MRID# 47976403
MRID# 47976402
MRID# 47976401
Developmental Toxicity Study in Rabbits
Pilot Developmental Toxicity Study in Rabbits
14-day Tolerability Study in Non-pregnant Rabbits
OPPTS 870.3700

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Tetrahedron Incorporated
1414 Key Highway, Suite B
Baltimore, MD 21230

Principal Reviewer: Henry Spencer, PhD — *Henry Spencer, Ph.D.* — Date: August 1, 2010

Secondary Reviewer: Isabel Mandelbaum — *Isabel Mandelbaum, Ph.D., D.A.B.T* — Date: 8/7/10

Tetrahedron Program Manager: Nasrin Begum — *Nasrin Begum, Ph.D.* — Date: 8/13/10

Quality Control: — *Daniel Ewald* — Date: 8-13-10

Contract Number: EP-W-13001
Work Assignment No.: WA-0-01

Task No.: 1-1-3
EPA WAM/Reviewer: Myron Ottley/Kit Farwell

This review may be altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Kit Farwell, D.V.M **Signature:** Kit Farwell
Registration Action Branch 7, Health Effects Division (7509P) **Date:** 10-26-2010
EPA Work Assignment Manager: Stephen Dapson, Ph.D. **Signature:** Stephen C Dapson
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 10/20/0010
Template version 02/06

TXR#: 0055337

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity in Rabbits by Gavage; OPPTS 870.3700b [§83-3] (non-rodent); OECD 414.

PC CODE: 600016 **DP BARCODE:** D376196

TEST MATERIAL (PURITY): Ethylenethiourea (ETU) (99.4%)

SYNONYMS: 2-Imidazolidinethione

CITATION: Munley, S.M. (2010). Ethylenethiourea (ETU): Developmental Toxicity Study in Rabbits. E .I. du Pont de Nemours and Company, Newark, Delaware. Laboratory report number: DuPont-17807-843. January 14, 2010. Unpublished. MRID 47976403.

Munley, S.M. (2010). Ethylenethiourea (ETU): A 14-Day Tolerability Study in Non-Pregnant Rabbits E. I. du Pont de Nemours and Company, Newark, Delaware. Unpublished. MRID 47976401.

Munley, S.M. (2010). Ethylenethiourea (ETU): Pilot Developmental Toxicity Study in Rabbits. E.I. du Pont de Nemours and Company, Newark, Delaware. Unpublished. MRID# 47976402.

SPONSOR: ETU Task Force: BASF Corporation; Dow AgroSciences LLC.; E.I. du Pont de Nemours and Company; United Phosphorus, Inc.

EXECUTIVE SUMMARY: In a rabbit developmental toxicity study (MRID 47976403) Ethylenethiourea (99.4% a.i., Sigma–Aldrich Corporation, Lot 00721KH) was administered to groups of 22 Hra: (NZW) SPF female rabbits per dose level by gavage on gestation days (GD) 7-29 as a formulation in 0.5% aqueous methylcellulose (Sigma, (Lot 017K0052) at dose levels of 0, 5, 15, or 50 mg/kg/day.

There were no deaths in the study. Body weight gains were reduced over the length of the study by 7% and 30% in the top two dose groups when compared to controls. The absolute body weights on day 29 for the mid- and high-dose groups were only reduced -2% and -4% of controls, respectively. Food consumption was also reduced by 7% and 14 % in these 2 groups. Thyroid weights were somewhat elevated when compared to controls, but these differences did not reach statistical significance. There was an increased incidence of discolored (dark) thyroid glands in the middle dose (2/21) and top dose (3/22), and the report of an enlarged gland in the middle dose

group.

The NOAEL for maternal toxicity is 15 mg/kg/day. The LOAEL for maternal toxicity is 50 mg/kg/day, based on reduced body weight gain of -30%, which corresponded to a decrease of -4% for absolute body weight. Additionally, a lowered food consumption of -14% occurred over the length of the study.

Fetal weights in the high-dose group were reduced -9% compared to controls. Two fetuses in separate litters of the high-dose group had domed heads and one of these fetuses had hydrocephalus. These effects may be due to treatment because hydrocephaly is a recognized effect of ETU treatment in rats.

The NOAEL for developmental toxicity is 15 mg/kg/day. The LOAEL for developmental toxicity is 50 mg/kg/day based upon reduced fetal weights. Also occurring at this dose were domed heads (2 pups) and hydrocephaly (1 pup).

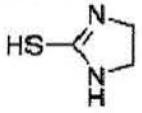
This developmental toxicity study in the rabbit is classified **ACCEPTABLE/GUIDELINE** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700b; OECD 414) in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided

MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Ethylenethiourea (ETU)

Description:	White powder with lumps
Lot/batch #:	Lot 00721KH
Purity:	99.4 % a.i.
Compound stability:	Up to 5 days at room temperatures as the formulation
CAS # of TGAI:	96-45-7
Structure:	

2. **Vehicle:** 0.5% aqueous methylcellulose: Lot #017K0052; Purity was not stated.

3. **Test animals:**

Species:	Rabbit
Strain:	NZW Hra: SPF
Age/weight at study initiation:	The females were from 5.5 to 6.0 months of age and weighed from 2709- 3662 g.
Source:	The supplier was Covance, located in Denver, Pa.
Housing:	The test animals were housed individually in stainless steel cages
Diet:	The diets provided were approximately 125 g of Certified Rabbit LabDiet® 5322, *ad libitum*, presented each day starting at arrival in the laboratory
Water:	Tap water was provided *ad libitum*
Environmental conditions:	**Temperature:** 61-72 °F **Humidity:** 30-70% **Air changes:** Not provided in the report

Photoperiod: 12 hrs dark/12 hrs light with the light provided as fluorescent illumination.

Acclimation period: The test animals were received as bred at gestation day 1 and quarantined for 3 days before the study was started.

B. STUDY DESIGN:

1. **In life dates:** Start: October 19, 2008; End: November 20, 2008

2. **Animal assignment:** Animals were randomly assigned by using a computerized randomization procedure to produce a homogenous weight distribution in the test dose groups noted in Table 1.

TABLE 1. Study design

Test group	Conc. in diet (mg/kg/day)	Gavage Dose to animal – GD Days 7-29 (mg/kg/day)	# Males	# Female
Control (Group 1)	0	0	0	22
Low (Group 2)	0	5	0	22
Mid (Group 3)	0	15	0	22
High (Group 4)	0	50	0	22

3. **Dose selection rationale:** The dose levels to be tested were selected based on the results from 2 different studies including:

1). A 14-day tolerability study in non-pregnant rabbits (see Appendix A for a review of MRID 47976401), where gavage administration of 400 mg/kg/day and 200 mg/kg/day resulted in decreased survival, significant body weight loss, low and or no food intake, diarrhea, and fur staining.

2). A pilot developmental toxicity study in rabbits (see Appendix B for a review of MRID 47976402), where gavage administration of 50 to 200 mg/kg/day resulted in the need for euthanasia at the highest dose prior to study end and slight tissue effects were noted in one female at 100 mg/kg. The lowest dose group (50 mg/kg/day) had lowered food consumption and reduced body weight gain, resulting in the use of that dose as the highest dose level in the present developmental toxicity study.

4. **Diet preparation and analysis:** A normal rabbit diet (Certified Rabbit LabDiet® 5322) containing no test material and weighing approximately 125 grams was presented daily to each animal. The gavage test doses were prepared fresh at least every 5 days by mixing appropriate amounts of test substance with methylcellulose to make a 0.5% aqueous solution; test solutions were stored at room temperature. Test substance homogeneity and concentrations were determined at the beginning and end of the dosing periods. Stability was tested in previous studies and found to be valid for 10 days at room temperature.

Results:

Homogeneity analysis: Homogeneity was examined for 4 different concentrations of dose samples. Dose levels of 10, 20, 30, and 40 mg/ml were sampled at a top, middle, and bottom site in each dose sample. The 10 mg/ml samples ranged from 10.4 mg/ml (top) to 9.86 mg/ml (middle) to 11.0 mg/ml (bottom). The 40 mg/ml sample ranged from 45.1 mg/ml (top) to 42.9 mg/ml (middle) to 43.8 mg/ml (bottom). Mean values of the 2 samples were 10.4±0.6 mg/ml (10 mg/ml sample) and 43.9±1.1 mg/ml (40 mg/ml sample). The measured samples at 10, 20, 30, and 40 mg/ml varied no more than 5%, 3%, 2%, and 3%, respectively, from their nominal target concentrations. These homogeneity data were obtained from data submitted in MRID 47976402 and were determined on samples prepared on July 11, 2008.

Stability analysis: Samples made on July 11, 2008 were also used to determine the stability of the mixtures. By using only 10 mg/ml and 40 mg/ml samples stored for 10 days at room temperature and resuspending the mixtures before sampling the top, middle, and bottom, determination of the 2 samples provided data which gave a mean value of 10.3±0.5 and 40.9±2.1 mg/ml for the 10 and 40 mg/ml samples, respectively. The data indicated that both the homogeneity and stability of the suspensions were adequate for the study.

Concentration analysis: Test doses were sampled prior to animal dosing and a short time prior to end of dosing for determination of dosing concentrations. The dose mixtures tested were 1, 3, and 10 mg/ml. Determinations from both dates were reasonably close and suggested that the animals did receive the appropriate dosing. Mean values reported for the first date for the 1, 3 and 10 mg/ml samples were 1.08±0.03, 3.16±0.06, and 10.8±0.2 mg/ml, respectively. Values reported for the second date were 1.04±0.05, 3.08±0.04, and 10.4±0.1 mg/ml, representing the 1, 3, and 10 mg/ml doses, respectively. These mean values of multiple determinations (2) were all within 5% or less than the nominal values.

Statistics:

Statistical analyses were conducted on a large number of parameters and were conducted in a step-wise manner to establish or rule out a significance at a $p < 0.05$ level. A list of parameters and the various statistical methods used is presented in the following table obtained from p. 20 of the report. This is a very meticulous but adequate and acceptable method of statistical analysis.

Table 2. Statistical analyses			
		Method of Statistical Analysis	
Parameter	Preliminary Test	If Preliminary test is not significant	If Preliminary test is significant
Maternal wt. **Maternal wt. change** **Maternal food consumption** **Corpora lutea** **Implantations per litter** **Live fetuses per litter** **Dead fetuses per litter** **Resorptions per litter**	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Incidence of pregnancy **Maternal mortality** **Females with total resorptions** **Abortions**	None	Sequential application of the Cochran-Armitage test	
Incidence of fetal alterations	None	Exact Mann-Whitney with a Bonferroni-Holm adjustment	
Fetal weight (Covariates: litter size, sex ratio) **Sex ratio (Covariate: litter size)**	Levene's test for homogeneity and Shapiro-Wilk test for normality	Analysis of covariance and Dunnett-Hsu	Non-parametric analysis of covariance

1. If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, the Kruskal-Wallis test was followed by Dunn's test.
2. If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact test with a Bonferroni correction was used.
3. A normalizing, variance stabilizing transformation was used as needed.

For litter parameters, the proportion of affected fetuses per litter or the litter mean was used as the experimental unit for statistical evaluation.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected twice daily during gestation day(s) (GD) 7-28 and once on GD 29 for signs of toxicity and mortality. Observations were made in 2 segments: the quarantine and pretest segment covering GD 4-6 and the second segment as the testing period covering GD 7-29. Observations in the pretest segment were made one time each day.

1b. Clinical examinations: Clinical examinations were conducted in the pretest period on GD 4, twice daily on GD 7-28, and once on GD 29.

2. Body weight: Animals were weighed on GD 4 and on testing days GD 7-29.

3. Food consumption and compound intake: Food consumption for each animal was determined daily on GD 4-29 and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency was not provided in the report.

4. Hematology and clinical chemistry: Blood was collected at termination for possible future

hormonal determinations. Other blood determinations were not carried out in this study.

5. **Sacrifice and pathology**: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the thyroids were collected for determination of organ weights.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** With the exception of intestinal effects which included diarrhea and lack of feces which were reported in the preliminary studies, clinical signs of toxicity were lacking compared to controls. Only at the top two doses was diarrhea found and only in one animal in each dose. Hair loss was reported in 2/22 controls as early as 8 days into treatment and 2/22 animals at 50 mg/kg/day on days 15 through 29 of the study. These effects are not considered to be definitely compound related

2. **Mortality:** Most of the treated females completed the study to sacrifice. One animal in the 5 mg/kg/day and one in the 15 mg/kg/day dose level were euthanized for medical reasons unrelated to treatment, probably inner ear infection and abortion, respectively.

B. BODY WEIGHT AND WEIGHT GAIN: Body weight gains were statistically significantly lower in the top two dose levels when compared to controls by the end of the study at day 29. Only a slight reduction in body weight gain is seen for the study at the 5 mg/kg/day dose level. The absolute body weights on day 29 for the mid- and high-dose groups were -2% and -4% of controls, respectively.

TABLE 3. Average body weights and body weight gains during treatment [a]

Dose rate (mg/kg/day)	Group mean weight (grams±SD) (n)				Mean weight gain (grams±SD) (n)	
	Week 1 Day 7	Week 2 Day 14	Week 3 Day 21	Week 4 Day 29	Day 7–29	Net body weight change Day 29 [b]
0	3168.4 ±247.1 (n=22)	3297.4 ±248.0 (n=22)	3402.8 ±257.6 (n=22)	3583.5 ±266.4 (n=22)	415.0g ±56.4 (n=22)	-66.08 ±55.87 (n=22)
5	3143.8 ±187.7 (n=22)	3255.4 ±177.1 (n=22)	3361.7 ±169.1 (n=21)	3516.0 ±196.1 (n=21)	390.7 ±91.5 (n=21)	-69.63 ±113.81 (n=21)
15	3201.2 ±251.1 (n=21)	3310.2 ±278.5 (n=21)	3392.6 ±275.2 (n=20)	3524.2 ±266.6 (n=20)	344.9* ±84.9 (n=20)	-77.08 ±99.13 (n=20)
50	3139.5 ±201.9 (n=19)	3191.8 ±237.7 (n=19)	3284.9 ±285.1 (n=19)	3430.5 ±289.5 (n=19)	291.1* ±162.3 (n=19)	-136.12 ±133.39 (n=19)

[a] Data obtained from pages 29-32 of the study report.

[b] Net body weight (body weight minus the gravid uterus weight) on Day 29 minus the net body weight on day 7.

*Statistically different ($p < 0.05$) from the control.

C. FOOD CONSUMPTION: Food consumption appeared to cycle in the 5 mg/kg/day dose level as noted in the values at 14 and 21 days. The low values seen at 7 days became lower for the rest of the study in both the 15 and 50 mg/kg/day levels, so that by the end of the study at day 29 the values representing the entire 22 days are statistically significantly less than the control (-7% and -14% of control values for the 15 and 50 mg/kg/day groups. respectively). The lowest dose also reported increasingly lower food consumption values by the end of the study. However, these lower levels did not quite reach a statistically significant level compared to controls.

Table 4. Food consumption at weekly intervals [a]

mean gram food/animal/day (±SD)
(n)

Dose Rate (mg/kg/day)	Day 7-8	Day 13-14	Day 20-21	Day 27-28	Day 28-29	Day 7-29
0	125.6±2.3 (n=22)	124.8±2.5 (n=22)	126.1±2.1 (n=22)	118.8±17.8 (n=22)	121.9±11.4 (n=22)	124.3±2.9 (n=22)
5	120.0±17.4 (n=22)	116.2±27.1 (n=22)	124.4±4.6 (n=21)	109.4±24.7 (n=21)	109.7±27.2 (n=21)	118.9±7.4 (n=21)
15	117.5±18.8 (n=21)	107.7±39.1 (n=21)	123.8±8.7 (n=20)	110.0±22.9 (n=20)	108.9±26.8 (n=20)	115.6*±8.9 (n=20)
50	108.0±26.9 (n=19)	92.3±42.7 (n=19)	112.3*±28.6 (n=19)	98.5±36.4 (n=19)	100.1±37.8 (n=19)	107.2*±17.2 (n=19)

[a] Data obtained from pages 33 and 34 of the study report.
* Nonparametric comparison to control (Dunn's) significant at $p<0.05$.

D. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The main organ weight changes were those of the thyroid gland and are noted below in the following table. The mean thyroid weights were increased about 9 to 13% above controls in the treated groups while the relative to final body weights were increased from 11 to 14%.

Table 5. Mean final body and organ weights for maternal rabbits

Group	1	2	3	4
Dose rate (mg/kg/day)	0	5	15	50
Body weight (grams±SD) (n)	3583.5 ±266.4 (n=22)	3516.0 ±196.1 (n=21)	3511.0 ±266.8 (n=21)	3415.1 ±276.4 (n=22)
Thyroid weight (grams±SD) (n)	0.223 ±0.053 (n=22)	0.243 ±0.054 (n=21)	0.252 ±0.062 (n=21)	0.242 ±0.059 (n=22)
Thyroid gland wt relative to final body weight x 100 (±SD) (n)	0.0062 ±0.0014 (n=22)	0.0069 ±0.0014 (n=21)	0.0072 ±0.0020 (n=21)	0.0071 ±0.0016 (n=22)

2. **Gross pathology:** Discoloration of the thyroid gland was reported in the top 2 dose groups.

Table 6. Incidences of gross observations in female rabbits

	Females			
Group	1	2	3	4
Dose rate (mg/kg/day)	0	5	15	50
Number of animals/group (n)	22	22	22	22
Stomach				
No visible lesions	0	0	0	0
Discoloration Red, glandular	0	0	1	0
Thyroid Gland				
Number of visible lesions	0	0	0	0
Discoloration Dark	0	0	2	3
Large	0	0	1	0
Uterus				
Number of visible lesions	0	0	0	0
Whole Body				
No visible lesions	22	22	19	18

3. **Microscopic pathology:** There were no reported microscopic evaluations of gross findings.

4. Cesarean section data:

Early resorptions were increased at the 15 mg/kg/day dose level. However, there was not a significant increase at the highest dose level.

Fetal weights for combined sexes were lower in both the middle and highest doses when compared to controls and reached statistical significance. Each total of both male and female fetuses was slightly; though not statistically significantly lower when compared to controls.

Table 7. Reproductive outcome [a]

Group	1	2	3	4
Dose rate (mg/kg/day)	0	5	15	50
Number of animals/group	22	22	22	22
Not pregnant (%)	0	0	0	3 (13.6)
Died/ Killed	0	0	0	0
Survived to scheduled kill	0	0	0	3
Pregnant (%)	22 (100.0)	22 (100.0)	22 (100.0)	19 (86.3)
Died/ Killed/ Aborted	0	1	1	0
With total resorptions	0	0	0	0
With live fetus at scheduled kill	22	21	20	19
Corpora lutea (mean±SD) (n)	8.8±1.2 (n=21)	8.4±1.9 (n=21)	8.0±2.3 (n=20)	8.8±1.8 (n=19)
Implants (mean±SD) (n)	8.5±1.3 (n=22)	8.3±1.9 (n=21)	8.1±2.2 (n=20)	8.4±1.6 (n=19)
Total resorptions (mean±SD) (n)	0.09±0.29 (n=22)	0.10±0.30 (n=21)	0.50**±0.69 (n=20)	0.47±0.77 (n=19)
Early resorptions (mean±SD) (n)	0.00±0.00 (n=22)	0.05±0.22 (n=21)	0.30**±0.57 (n=20)	0.26±0.65 (n=19)
Late resorptions (mean±SD) (n)	0.09±0.29 (n=22)	0.05±0.22 (n=21)	0.20±0.52 (n=20)	0.21±0.54 (n=19)
Dead fetuses	0 (n=22)	0 (n=21)	0 (n=20)	0 (n=19)
Live fetuses (mean±SD) (n)	8.4±1.1 (n=22)	8.2±1.9 (n=21)	7.6±2.2 (n=20)	7.9±1.7 (n=19)
Male fetuses (mean±SD) (n)	4.4±1.0 (n=22)	3.8±1.5 (n=21)	4.0±1.5 (n=20)	4.2±1.9 (n=19)
Female fetuses (mean±SD) (n)	4.0±1.3 (n=22)	4.4±1.1 (n=21)	3.6±1.6 (n=20)	3.7±1.6 (n=19)
Fetal weights (mean±SD) (n)	41.06±3.77 (n=22)	40.73±3. (n=21)	39.92*±5.88 (n=20)	37.28*±4.24 (n=19)
Male weights (mean±SD) (n)	41.44±3.84 (n=22)	40.89±2.90 (n=21)	39.92±5.95 (n=20)	38.04±4.22 (n=19)
Female weights (mean±SD) (n)	40.75±4.40 (n=22)	40.57±3.86 (n=21)	39.08±5.44 (n=19)	36.22±4.93 (n=19)
Sex ratio [b] (mean±SD) (n)	0.52±0.13 (n=22)	0.46±0.11 (n=21)	0.53±0.18 (n=20)	0.52±0.18 (n=19)

[a] Data were obtained from Table 7, pages 37-38 of the study report.
[b] Sex ratio = the number of male fetuses /total number of fetuses per litter
*p<0.05 for the Analysis of Covariance and Dunnett-Hsu test
**Indicates the nonparametric comparison to control (Dunn's) as significant at p<0.05.

5. Incidence of fetal malformations and variations

Two fetuses from separate litters in the high-dose group had domed heads and one of these fetuses had hydrocephaly. The registrant did not consider this effect to be treatment related, because hydrocephaly has been reported in historical control data. The study report included this paragraph:

> "For the thirteen studies for which historical control data were previously cited, there is one study conducted in 2001, which include one control fetus with hydrocephaly. In addition, there are 2 additional studies conducted in 1996 and 1998 in which there were 3 fetuses from 2 control litters and 5 fetuses from 5 control litters reported with distended brain lateral ventricles, which is considered to be a slightly less severe manifestation of internal hydrocephaly."

Although hydrocephaly has been noted to occur in a control fetus, hydrocephaly has also been reported in numerous developmental toxicity studies in rats treated with ETU. Because the hydrocephaly and domed heads occurred only in the high dose group in this study, and because hydrocephaly is a well know effect with ETU in rat developmental studies, hydrocephaly and domed heads are considered possibly treatment related effects in this study.

Other variations and malformations occurred sporadically in the different dose groups with no dose response and were not attributed to treatment.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigators believed that there were no maternal clinical observations that could be attributed to the test material. The investigators reported that there were maternal body weight changes that were attributed to 15 and 50 mg/kg/day of the test material. Maternal food intake was reduced in all dose groups, but only of significance at 15 and 50 mg/kg/day. Gross findings in various organs of the does were limited to the thyroid gland, including a non-statistical increase in gland weights. The glands were found to be discolored/darkened in 2 and 3 does at 15 and 50 mg/kg/day, respectively. The investigators considered that these effects might be test material-related. The investigators reported that there were statistically significant reductions in fetal body weights at the top two dose levels. The reductions were 3% and 9% less than the control mean. Of significance was the fact that the reductions coincided with the lower body weight gains, and lower food intakes of the does. The weight changes for the middle dose group were said to be within the historical values for the laboratory and thus were not considered to be an adverse effect.

The investigators concluded that "there were no test substance-related increases in the incidences of fetal malformations or variations at any level tested". The 2 instances of fetuses with a condition of "domed head" were not attributed to treatment because they were reportedly within the historical control range.

The investigators reported the maternal NOAEL to be 5 mg/kg/day based on reduced food intake and reduced body weight gains produced at 15 and 50 mg/kg/day. The fetal NOAEL is 15

mg/kg/day based on a LOAEL of 50 mg/kg/day with reduced fetal weight. The NOAEL for fetal malformation is 50 mg/kg/day based on the lack of increased incidences of fetal malformation or variation at any dose level compared to controls. "The results of this study indicate that the test substance is not uniquely toxic to the rabbit conceptus".

B. REVIEWER COMMENTS:

Body weight gains for the mid- and high-dose does were -17% and -30% of controls, respectively, for days 7-29. The absolute body weights on day 29 for the mid- and high-dose groups were -2% and -4% of controls, respectively.

Two fetuses from separate litters in the high-dose group had domed heads and one of these fetuses had hydrocephaly. Although hydrocephaly can occur spontaneously, the domed heads and hydrocephaly are considered treatment-related effects because hydrocephaly is a common finding in developmental toxicity studies in rats with ETU.

C. STUDY DEFICIENCIES:

The study was reasonably well conducted. There were no major deficiencies in reporting the data.

Appendix A: Pilot Study MRID 47976401

Citation: Ethylenethiourea (ETU): A 14-Day Tolerability Study in Non-Pregnant Rabbits, by Susan M. Munley, completed January 27, 2010 in the laboratory of E. I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, Delaware, 19714, unpublished, MRID 47976401.

Methods: The study report authors provided signed statements of No Data Confidentiality, Good Laboratory Practice Compliance, and Certification (Quality Assurance). The study was started on June 2, 2008 and ended on June 23, 2008. The test material was ETU, obtained from Sigma-Aldrich, with purity of 99.4%. Test material was suspended in 0.5% methylcellulose. Concentration, homogeneity, and stability assessments were conducted near the beginning of the study and the results were within 5% of nominal values.

There were 3 does in each of 3 dose groups: 100 mg/kg/day 200 mg/kg/day, and 400 mg/kg/day. Dosing continued in each group for 14 days. Does were sacrificed on day 15 and given gross examinations. Examinations included: mortality, clinical observations, body weights, and food consumption.

Results: All 3 does in the 100 mg/kg/day group survived to termination. On day 5, 1 does had absent feces and did not. These signs had reversed to normal by day 6.

One doe in the 200 mg/kg/day group was euthanized on day 10 because of body weight loss, lack of food consumption, and clinical observations of absent feces, diarrhea, and stained fur. The remaining 2 rabbits survived to study end.

At 400 mg/kg/day only 1 rabbit survived to study end. The other 2 rabbits were euthanized on day 6 because of the same effects seen in the toxic rabbit at the next lower dose (200 mg/kg/day).

This range-finding study was classified acceptable/non-guideline.

Appendix B: Pilot Study (MRID# 47976402)

Citation: Ethylenethiourea (ETU): Pilot Developmental Toxicity Study in Rabbits, by Susan M. Munley, completed January 14, 2010. Performed at E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, Delaware for The ETU Task Force: BASF Corporation, Dow AgroSciences LLC., E.I. du Pont de Nemours and Company, United Phosphorus, Inc., unpublished. MRID# 47976402.

Methods: The study report authors provided signed statements of No Data Confidentiality, Good Laboratory Practice Compliance, and Certification (Quality Assurance).

The test material was ethylenethiourea, (ETU), 2- imidizoladinthione (CAS Number 96-45-7) and was reported to be 99.4% pure. The in-life dates were: start - July 13, 2008, end - August 8, 2008. This study used 5.5-6 month old animals obtained from Covance, Pa. Six or seven nulliparous, time-mated Hra:(NZW)SPF white rabbits per group received ethylenethiourea (ETU) obtained from Sigma-Aldrich Corp. Lot 00721KH. ETU was suspended in a 0.5% commercial solution of methylcellulose and given as 5 ml/kg by gavage once a day at dose levels of 200, 150, 100, or 50 mg/kg/day. Dosing samples were analyzed for concentration, homogeneity, and stability for the study and were within range of target values for all dose levels. The animals were observed during day 7 through day 28 of gestation for clinical signs, absent feces, diarrhea, body weight loss, and lack of food intake.

The study animals were kept on a 12 hour light-dark cycle and provided PMI® Nutrition International, LLC. Certified Rodent LabDiet ® 5002 (pellets), *ad libitum*, and tap water, *ad libitum*, from United Water Delaware. The laboratory room temperature was set at 18-26°C with humidity of 30%-70%. The temperature and humidity data were omitted from the study report. Does which aborted, or were in extremis, were euthanized before sacrifice day 29 and given gross examinations.

Results: All does in the lowest 2 dose groups (50 and 100 mg/kg/day) survived to the end of the study. Food consumption was lowered by 16% and 20% in these groups. When compared to the body weight gains of controls, the 50 and 100 mg/kg/day groups were only 46% and 35% of control values for the same test period.

Does in the 150 and 200 mg/kg/day groups were terminated between gestation days 18 and 23 because of excessive toxicity: marked reductions in food consumption, weight loss, and clinical signs of impending abortion which included perineal fur staining and red-stained floors.

Mean fetal weights in the 50 and 100 mg/kg/day groups were reduced in comparison to controls by 22% and 30%, respectively. The fetal body weight differences were the only statistically significant changes reported in the parameters tested for fetal toxicity which included: corpora lutea, implantations, live fetuses, dead fetuses, resorptions, and incidence of fetal alterations.

This range-finding study was classified acceptable/non-guideline.

RETURN THIS FORM TO
WAM, WHEN DER IS FINAL

EPA REVIEWER'S ASSESSMENT OF CONTRACTOR'S PERFORMANCE

ASSIGNMENT	CHEMICAL NAME:				
Study Type	*Accession# MRID*	*DER Completion Date*	*Date Received*	*EPA Reviewer Evaluation*	*Hours Spent Performing Secondary Review*
Rabbit developmental toxicity study	47976403	10/27/2010	8/2010	DER was adequately written.	12
*Quality- **1-Good***				*Reviewer Signature:*	
*Timely- **1-Excellent***				*Section Head Initials:*	

13544

R186733

Chemical Name: Ethylenethiourea

PC Code: 600016
HED File Code: 13000 Tox Reviews
Memo Date: 10/27/2010
File ID: 00000000
Accession #: 000-00-0136

HED Records Reference Center
11/2/2010